BMC Plant Biology

RESEARCH

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Genetic composition and heterozygosity of gibberellin-induced 2n gametes evaluated by SSR markers in *Populus*

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Abstract

Background The artificial induction of unreduced (2n) gametes, due to first division restitution (FDR) or second division restitution (SDR), is an important method to produce triploids in higher plants. It is crucial to evaluate the genetic composition and heterozygosity transmission of induced 2n gametes with different formation mechanisms.

Results Here, we produced 110 triploids by pollinating the female inflorescence of *Populus alba* × *P. glandulosa* with gibberellin-induced 2n pollen of *Populus bolleana*. The genetic composition and heterozygosity of the induced 2n pollen were inferred from SSR marker analysis of the triploid offspring, using 27 primer pairs distributed across nine *Populus* chromosomes. This revealed that, among the 110 hybrid triploids, 70 triploids did originate from FDR-type 2n pollen and the other 40 from SDR-type 2n pollen. The FDR-type and SDR-type 2n pollen transmitted a substantially different level of parental heterozygosity (0.7545 and 0.3167, respectively) in *P. bolleana*. Additionally, compared with the low coefficient of variation (CV = 12.82%) for heterozygosity transmitted by FDR-type 2n pollen, there was much higher coefficient of variation (CV = 48.82%) for that transmitted by SDR-type 2n pollen.

Conclusions Our findings provide a valuable reference point for bolstering suitable applications of 2n gametes in future research, and can help foster new strategies of triploid breeding in plants.

Clinical trial nubmer Not applicable.

Keywords GA_3-induced 2n gametes, First division restitution, Second division restitution, Transmission of parental heterozygosity, SSR marker

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Background

Polyploidization via whole-genome duplication is a major force of evolution and diversification, especially in higher plants. Molecular analyses suggest that more than 90% of angiosperms are estimated to have undergone at least one or more polyploidization events during their evolution [1]. The hybridization of unreduced (2n) gametes with haploid gametes is thought to be the main source of polyploid formation [2, 3]. Many polyploid individuals have been generated by crossing artificially induced 2n gametes in *Citrus*, *Populus*, and *Eucalyptus*, as well as other plant genera [4–7]. Accordingly, it is essential to evaluate the genetic composition and respective heterozygosity of artificially induced 2n gametes.

Many plant species harbor 2n gametes [8] and these can be artificially induced by physical or chemical mutagens [9]. In general, the main mechanisms underpinning 2n gamete formation fall into four categories: first-division restitution (FDR), second-division restitution (SDR), postmeiotic restitution (PMR), and indeterminate meiotic restitution (IMR) [10]. In plants, previous studies have shown that 2n gametes are predominantly formed through FDR and SDR [6, 11, 12]. Whereas FDR-type 2n gametes result from failed spindle formation during meiosis I, leaving chromosomes unable to move to opposite poles, SDR-type 2n gametes occur when sister chromatids fail to separate during meiosis II [13, 14]. As such, significant differences arise in both the genetic composition and heterozygosity between FDR-type and SDR-type 2n gametes. Notably, the heterozygosity transmitted by FDR-type 2n gametes is around double that of the SDR-type [15–18]. Further, it is general knowledge that FDR-type 2n gametes with higher heterozygosity have been widely applied to improve polyploid traits [19]. Therefore, understanding the genetic composition and heterozygosity of 2n gametes is of paramount importance for their effective utilization in plant breeding programs.

Populus is a recognized model system for woody plant biology. In our previous work, we reported on meiotic restitution and 2n pollen induced by interfering the radial microtubule arrays (RMAs) during male meiotic cytokinesis with an exogenous gibberallic acid (GA_3) injection to male flower buds in *Populus bolleana* [20]. In the present study, we first produced triploids by crossing gibberellin-induced 2n pollen with the haploid female gametes of the hybrid *Populus alba* × *Populus glandulosa*. Next, the genetic composition and heterozygosity of GA_3-induced 2n pollen was investigated using a suite of SSR markers. Our empirical findings provide a timely reference for enhancing the applications of 2n gametes in future studies, with a view to helping devise new and better strategies for triploid breeding in woody plants.

Materials and methods

Plant materials

The male flowering branches of *P. bolleana* ($2n = 2x = 38$) were collected from Inner Mongolia Agricultural University, China. The female flowering branches of *P. alba* × *P. glandulosa* ($2n = 2x = 38$) were collected from Guan County, in Shandong Province. All sampled floral branches were pruned and cultivated in a greenhouse (10–20 °C) at Beijing Forestry University. No additional nutrients were added to the tap water supplied.

Triploid production by crossing GA_3-induced 2n pollen

When the pollen mother cells (PMCs) of *P. bolleana* were at the prophase II stage, the male flower buds were given seven injections with 10 μM GA_3 solution, to induce 2n pollen production [20]. Next, the stigmas of female flower buds of *P. alba* × *P. glandulosa* were pollinated with the resulting GA_3-induced 2n pollen. After pollination, the female flower branches were hydroponically grown in a greenhouse. About four weeks later, the progeny seeds had reached full maturity. Collected seeds were sown in nutrient trays ($54 \times 28 \times 10$ cm) with a depth of 5 cm to promote growth. When the surviving seedlings were approximately 25 cm in height, they were transplanted into the field.

Ploidy level detection by flow cytometry and somatic chromosome counting

Flow cytometry (BD FACSCalibur, USA) was used to determine the ploidy level of the progeny, by following methodology described by Zhou et al. [21] to verify the putative triploid. To confirm the ploidy level of each ensuing plantlet, somatic chromosome counting was carried out, as described by Tian et al. [4].

DNA extractions and simple sequence repeat (SSR) analysis

The DP320 DNA Secure Plant kit (Tiangen Biotech Co., Ltd. in Beijing, China) was used to extract the DNA from each stored leaf sample. The Sangon Biotech Co., Ltd. (Shanghai, China) synthesized all the SSR primer pairs used in this study, which were derived from three sources: (1) the SSR primers (i.e., starting with 'GCPM', 'ORPM') published by the International *Populus* Genome Consortium (IPGC; http://w.ornl.gov/sci/ipgc/ssr_resource.htm); (2) those SSR primers developed from the *P. tric hocarpa* genomic sequence (i.e., starting with 'LG') [22]; and (3) some SSR primers developed from the mRNA sequence data of *P. alba* in the laboratory (i.e., starting with 'PTSSR' and 'MB'). The SSR primer sequences were aligned with the genome of *P. trichocarpa* v4.1 (https://phytozome-next.jgi.doe.gov/info/Ptrichocarpa_v4_1),

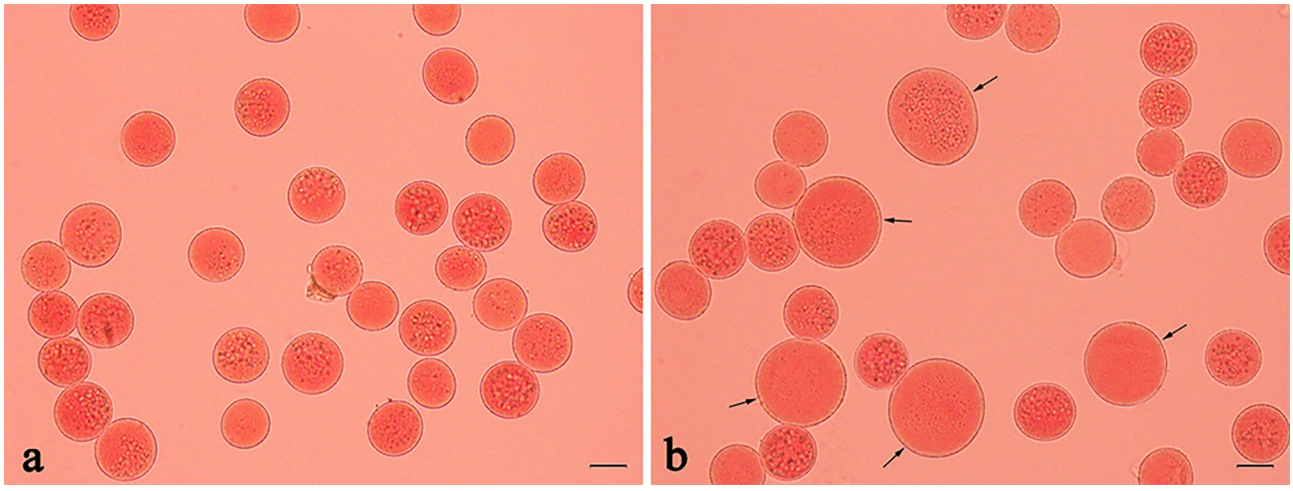

Fig. 1 Haploid and GA_3-induced 2n pollen in male flower buds of *P. bolleana*. (**a**) Haploid pollen from the control group. (**b**) GA_3-induced 2n pollen from the male buds given seven injections of 10 μM GA_3 (arrow). Bars = 20 μm

using BLAST, to determine the precise physical location of each SSR marker on the chromosomes [23, 24].

For the polymerase chain reaction (PCR), we used the fluorescently labeled TP-M13-SSR PCR method described by Schuelke [25], along with a sequence-specific reverse primer for the studied SSR marker, a forward primer with a universal M13 primer tail (5'-TGTAAAA CGACGGCCAGT-3') at the 5' end, and a universal M13 primer (fluorescently labeled with 6-carboxy-X-rhodamine, 6-carboxy-fluorescein, tetramethyl-6-carboxyrhodamine, or 5-hexachloro-fluorescein). All PCRs were carried out under these cycle conditions: 5 min at 94 °C; then 25 cycles of 30 s at 94 °C, 30 s at the optimal annealing temperature for each SSR marker, and another 30 s at 72 °C; then eight cycles of 30 s at 94 °C, 30 s at 53 °C followed by 30 s at 72 °C; and a final extension of 8 min at 72 °C. An ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA) was used to perform the capillary electrophoresis fluorescence-based SSR analyses. Finally, Gene Marker 1.75 software [26] was employed to analyze the allelic configuration of each sample from the obtained raw sequence data.

Heterozygosity analysis

We used POPGENE v1. 32 software [27] to calculate the observed values of heterozygosity (Ho). All statistical analyses were implemented in SPSS software (IBM SPSS Inc., Chicago, IL, USA).

Results

Triploid production by crossing GA_3-induced 2n pollen

When PMCs of sampled male flower buds were at prophase II in *P. bolleana*, they were given seven injections of 10 μM GA_3 to induce 2n production. The treated male flower buds were hydroponically cultured in a greenhouse until the catkins matured. Upon pollen release, GA_3-induced 2n pollen was collected from the treated male buds. In the control group, no 2n pollen grains were detected (Fig. 1a). GA_3-induced 2n pollen was collected from all surviving treated male buds (Fig. 1b).The frequency of GA_3-induced 2n pollen ranged from 5.92 to 20.64% (Table 1). Subsequently, the artifical induced 2n pollen were selected for hybridization with female gametes of *P. alba* × *P. glandulosa*.

Table 1 Crossing GA_3-induced 2n pollen with female gametes in *P. alba* × *P. glandulosa* to produce triploids

Group	Frequencies of induced 2n pollen (%)	Number of seeds	Number of seedlings	Number of triploids
1	5.92	22	17	1
2	9.33	107	78	1
3	14.84	46	40	1
4	12.37	370	224	1
5	13.75	126	97	2
6	15.24	420	342	3
7	9.46	530	431	4
8	12.13	2520	2054	21
9	20.64	6084	4716	76
10 (control)	0.00	300	241	0
Total		10,525	8240	110

Note: Group 1 and 2 represented the treatments given three injections of 10 μM GA_3 when the PMCs were at prephase II. Group 3 and 4 represented the treatments given five injections of 10 μM GA_3 when the PMCs at prephase II. Groups 5 to 9 represented the treatments given seven injections of 10 μM GA_3 when the PMCs at prephase II. Group 10 represented the treatment given five injections of distilled water when the PMCs at prephase II

From both the GA_3-induced 2n pollen and control pollen sources, a total of 10, 525 seeds were collected (Table 1). The number of seeds varied among treatment combinations due to differences in pollen quantity, female flower receptivity, and pollination efficiency. Of these, 8240 seeds were sown and became young seedlings. Based on the peaks measured by flow cytometry,

the seedlings were divided into diploids (Fig. 2a) or triploids (Fig. 2b). A total of 110 putative triploids were obtained. Follow-up somatic chromosome counting confirmed the diploids had a chromosome number of 38 (2n = 2*x* = 38) (Fig. 2c), and that all putative triploids were true triploids (2n = 3*x* = 57) (Fig. 2d). All the triploids originated from the cross combination with GA_3-induced 2n pollen, with no triploids detected from the cross with control pollen.

SSR marker screening

Although SSR markers can be used to effectively analyze the genetic composition of individual loci, recombination can lead to the mistaken origin of 2n gametes. Therefore, from 193 primer pairs we screened 27 pairs of SSR markers to identify the genetic composition of 2n gametes (Table 2). Selection was based on the fact that the allelic configuration in the male parent *P. bolleana* was heterozygous and differed from that of the female parent hybrid (*P. alba* × *P. glandulosa*). The specific information about of each SSR marker, such as their chromosome number, primer sequence, melting temperatures (Tm), motif

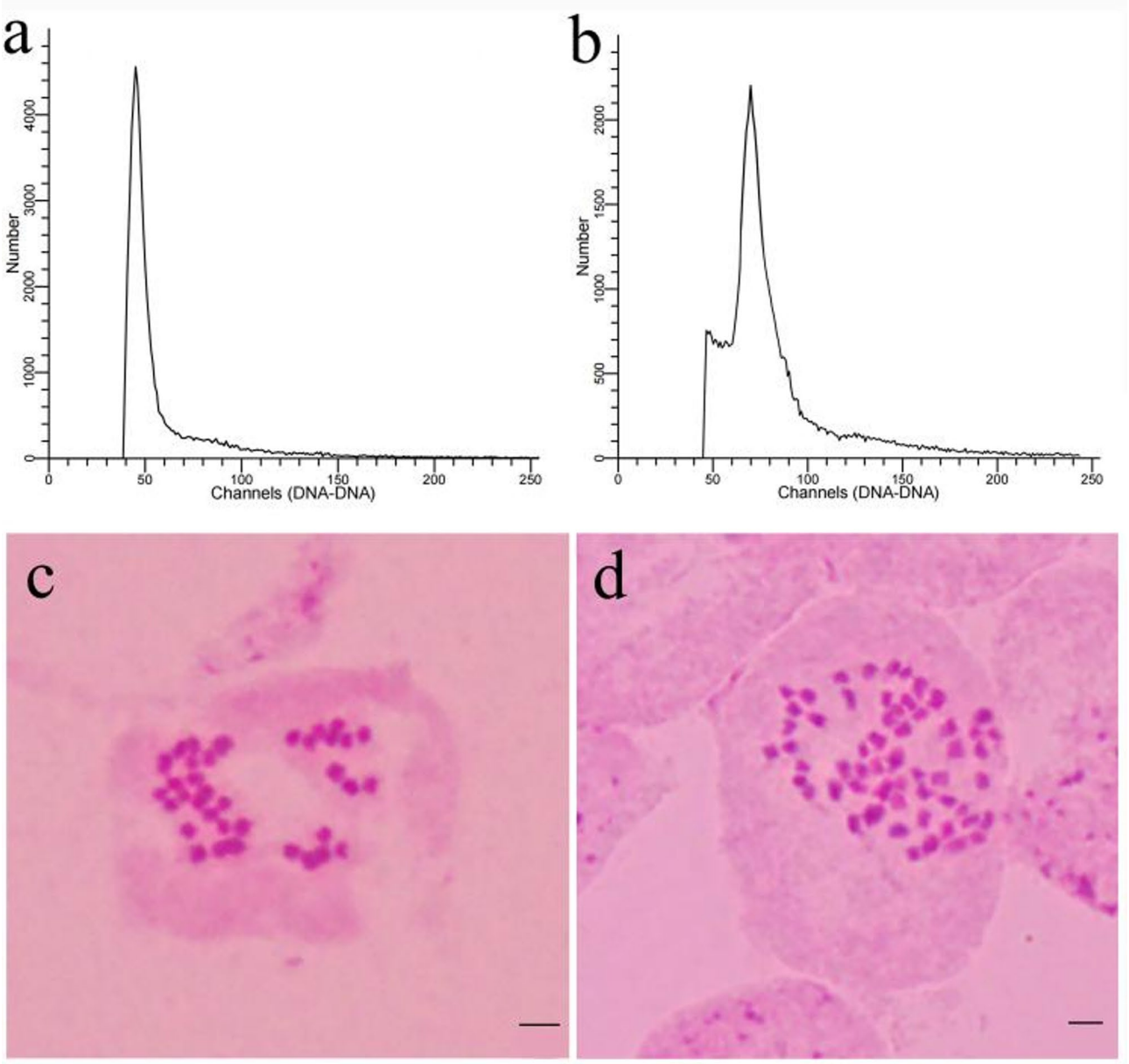

Fig. 2 Ploidy level analysis of progeny derived from the cross combinations with GA_3-induced 2n pollen and control pollen. Flow cytometry of (**a**) diploid and (**b**) triploid plants. Chromosome number of (**c**) diploid plants (2n = 2*x* = 38) and (**d**) triploid plants (2n = 3*x* = 57). Bars = 5.0 μm

Table 2 Information about the 27 pairs of SSR primers used for the detection of 110 triploids

Name	Chromosome no.	Primer sequence	Tm (°C)	Motif	Allele in the female parent	Allele in the male parent
GCPM-2151	03	Forward: TTCGTCATCGTTAATTTCAA Reverse: GTTGATTCATTGGGAAAATG	51.1 50.9	$[AT]_{14}$	155.4/165.4	163.5/169.5
LG-III	03	Forward: GGATATGTCTCCACAAAGGA Reverse: GTACTGTCTCCGATACTGCC	54.1 57.2	$[AC]_{10}$	155.4/165.5	163.7/169.6
PTSSR1566	03	Forward: GCGCTCTTCTTCCACGACTA Reverse: ACAAGCAGAACAAGTTAGCCCT	59.8 60.2	$[GCA]_{6}$	236.8/241.6	246.5/251.4
PTSSR1738	04	Forward: CTTCCTCGTTTGGCCCCTAA Reverse: ACCAGCTAGTTCGGGCTTTC	59.7 60.0	$[CTCTTC]_{5}$	288.5/299.8	282.8/288.3
MB71513	04	Forward: CCCGACAAGACAAATGTCAA Reverse: CAAAACGGGTTGTTTTTGCT	59.5 60.0	$[AGA]_{6}$	255.2/258.0	258.1/260.8
PTSSR730	07	Forward: TGTCTGGGAGATCTGCAGGA Reverse: CTGCCTCCTACCTCTCCCTT	60.0 60.0	$[GA]_{7}$	259.6/263.5	252.1/255.9
PTSSR2379	07	Forward: CTGCTTCACATCGATGCTGC Reverse: GTGCTGACCAGAGTTCTGCT	60.0 60.0	$[GTT]_{8}$	278.1/280.9	280.9/289.6
PTSSR2599	07	Forward: GGCTTTTGGATGCGGTCATC Reverse: TTGCTCCACAATCCCTGCAA	59.9 60.2	$[TG]_{15}$	158.2/162.4	152.9/158.2
PTSSR1518	09	Forward: CCAAAGACCAGCTTCAAGACC Reverse: AAGTGCAGCTCCCTCCTTTC	59.1 60.0	$[GAT]_{5}$	255.3/267.5	243.7/249.7
PTSSR1904	09	Forward: AACCAAACCAAACCAACGCG Reverse: GGGCGAATCTGCTACGATGA	60.5 60.0	$[TC]_{7}$	206.7/208.6	208.6/212.7
PTSSR1037	09	Forward: ACACGATCACACCCATGCTA Reverse: CAAGTGTGTGCCCTCTGGAA	59.1 60.2	$[GAA]_{6}$	289.9/294.2	291.5/294.2
PTSSR1042	09	Forward: CCAAATGAACAGCCACAGCC Reverse: TGTTGTGTTTGGCTCAATTGCT	60.0 59.8	$[AGC]_{5}$	285.7	285.7/288.5
PTSSR1359	09	Forward: GTGATGAATGCGGGATTGCC Reverse: TGTTTGGCTGAGCATTTGGC	60.0 60.0	$[CCA]_{6}$	165.6	162.8/168.6
PTSSR1543	09	Forward: ACAAGGAGAGAGAAACATAAGTTGT Reverse: GCGACCGAAAAAGTTTTGCC	58.2 59.2	$[TG]_{6}$	114.9	119.1/131.6
PTSSR1189	12	Forward: AATGGCTGTTCTCTCACGCA Reverse: CTGAGTGAGTGTCTGAGCCG	60.0 60.1	$[CCA]_{5}$	182.2/185.1	182.4/188.2
Ptr-12-SSR53	12	Forward: AGCAAGAACTTACAGCAAGC Reverse: CAACTTCTCCAATCTATGCC	52.7 52.2	$[TGC]_{24}$	230.6/243.4	224.5/230.7
PTSSR1539	12	Forward: CTCCAGTCTCCGCCGAAATT Reverse: GTGGATGACTGGGAGAAGGC	60.1 60.1	$[AAG]_{7}$	279.5	273.8/279.5
Ptr-13-SSR40	13	Forward: TGCTTATGGGTACAATGACA Reverse: GACCTCTGTAAACCCATTCA	51.9 52.1	$[TC]_{15}$	278/294.2	296.0/297.9
PTSSR1817	15	Forward: GGACTAAAAGGACCGGGTCG Reverse: CTCTCGCCTTCAGATCTCCG	60.1 59.7	$[TGAT]_{6}$	249.3/257.2	261.1/276.8
PTSSR1101	15	Forward: GCTCGATTTGGATCACCGGA Reverse: ACCTGCGCAATCTCATCCAT	60.2 59.8	$[CTCTC]_{5}$	236.4/241.5	246.6/251.6
PTSSR466	15	Forward: TCCTCTGCACCTTGTTCTCTC Reverse: GTTACTGTTCAGGAGCCCCA	59.4 59.3	$[CTT]_{8}$	389.7/395.6	389.7/392.5
PTSSR717	15	Forward: GTGGAGAAATGGGGAGTGGG Reverse: CCATGACAGCAAGACACCCT	59.7 60.0	$[TA]_{8}$	144.5	147.6/150.7
PTSSR661	17	Forward: GCAAACTCGATGAGGACCCA Reverse: CCACCAACACTTCCTCTCCG	60.0 60.3	$[AGA]_{6}$	265.0	265.0/267.7
PTSSR2535	17	Forward: TTCGAGCTTGATCGGATCGG Reverse: TGAAAACCCAAACCCAACTGA	60.0 58.2	$[GA]_{6}$	150.3	143.8/155.2
PTSSR355	17	Forward: GCCCGGTTTGGAGACCTAAA Reverse: ACTTTGCAGGTCTATTTGGCC	60.0 58.8	$[AG]_{8}$	171.9/200.8	179.2/183.9
PTSSR1245	19	Forward: TGGCGGAGAGGAATACCAGA Reverse: CCCATTGATTCTTCCAAGTACACC	60.0 59.6	$[AAG]_{6}$	246.5/249.4	254.8/257.5
PTSSR1853	19	Forward: AGTTTCCTCGGTCACAGCTG Reverse: GGAGGACACTACGCCCTTTT	60.0 60.0	$[TTAT]_{5}$	286.0/288.6	286.1/293.6

sequences, and physical locations in the parents, are presented in Table 2. Finally, these 27 pairs of SSR markers were distributed across nine chromosomes of *Populus.*

The 27 polymorphic SSR primers could be reliably classified into four types. In the first type, both parents were heterozygous and had distinct alleles; taking the primer PTSSR1101, as an example, the parents *P. alba* × *P. glandulosa* 'YXY' and *P. bolleana* 'XJY' were heterozygous with "ab" and "cd" genotypes, respectively (Fig. 3a). In the second type, both parents were heterozygous but shared one allele. Taking the primer PTSSR1904, as an example, the parents 'YXY' and 'XJY' were heterozygous with "ab" and "bc" genotypes, respectively, and they shared one allele at the PTSSR1904 locus (208.6 bp) (Fig. 3b). In the third type, the female parent 'YXY' was homozygous, but male parent 'XJY' was heterozygous; taking the PTSSR717 as an example, 'YXY' was homozygous with an "a" genotype and XJY' was heterozygous with a "bc" genotype (Fig. 3c). In the fourth type, 'YXY' was likewise homozygous with an "a" genotype, while 'XJY' was heterozygous with an "ab" genotype, and they shared one allele at the PTSSR1539 locus (279.5 bp) (Fig. 3d).

Genetic composition of GA_3-induced 2n pollen in *P. bolleana*

The 27 pairs of primers screened could be used to classify the FDR-type and SDR-type triploids into three categories, these corresponding to three genotypes of the 2n male gametes: "ab", "aa", and "bb"-type 2n gametes (Fig. 4). Among them, "aa" and "bb"-type 2n gametes only harbor half of the heterozygous information of male parent *P. bolleana*. By contrast, the "ab"-type 2n gametes contain all the heterozygous information inherited from *P. bolleana*. Based on the results for the 27 SSR primers in the 110 triploids, if more than half of the primers showed heterozygous paternal allele information, the triploid plant was deemed to have originated from an FDR-type 2n gamete. Conversely, if more than half of the primers displayed homozygous paternal allele information, the triploid plant is considered to have come from an SDR-type 2n gamete. According to this defining criterion the genetic composition of 2n gametes for the 110 triploids was determined and presented in Table 3. Of the 110 triploids, 70 originated from FDR-type 2n pollen and the other 40 from SDR-type 2n pollen, which respectively accounting for 63.64% and 36.36% of the total number of triploid plants.

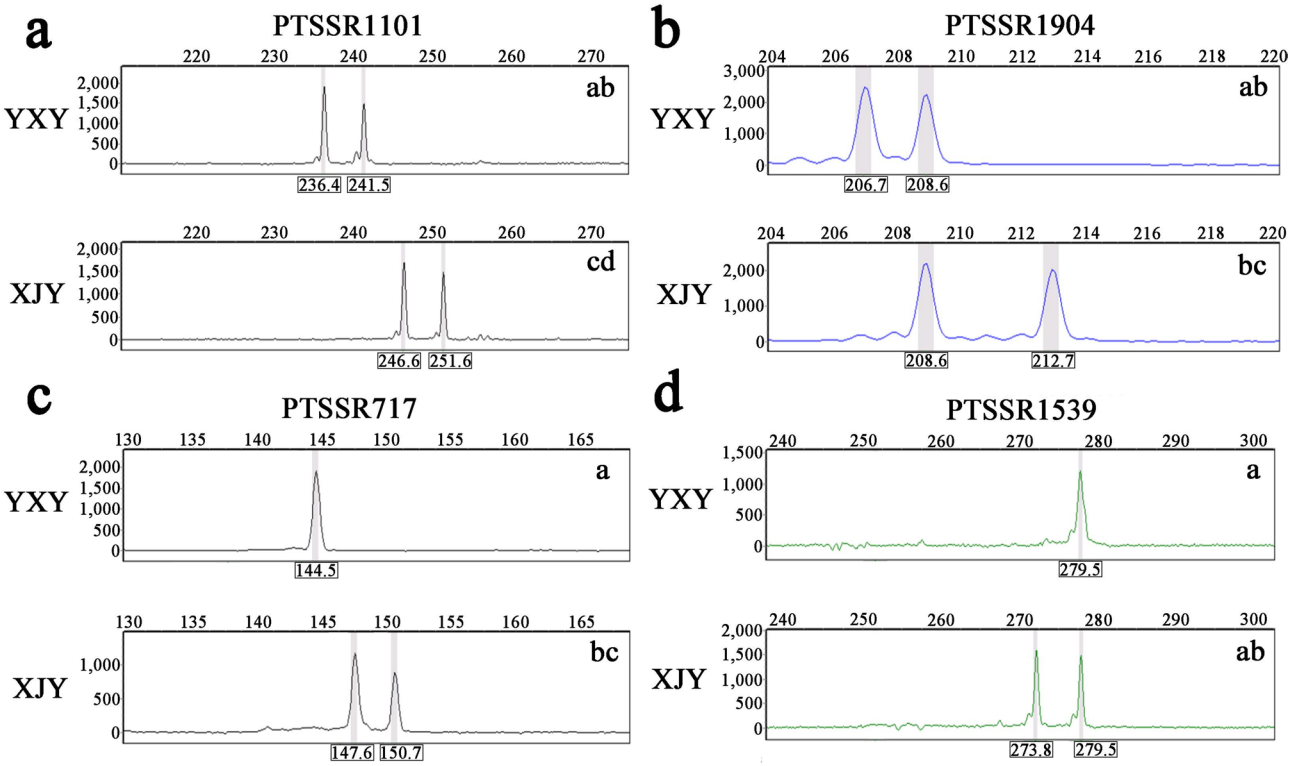

Fig. 3 Capillary electrophoresis was used for the SSR markers selection in the female parent of *P. alba* × *P. glandulosa* 'YXY' and the male parent of *P. bolleana* 'XJY'

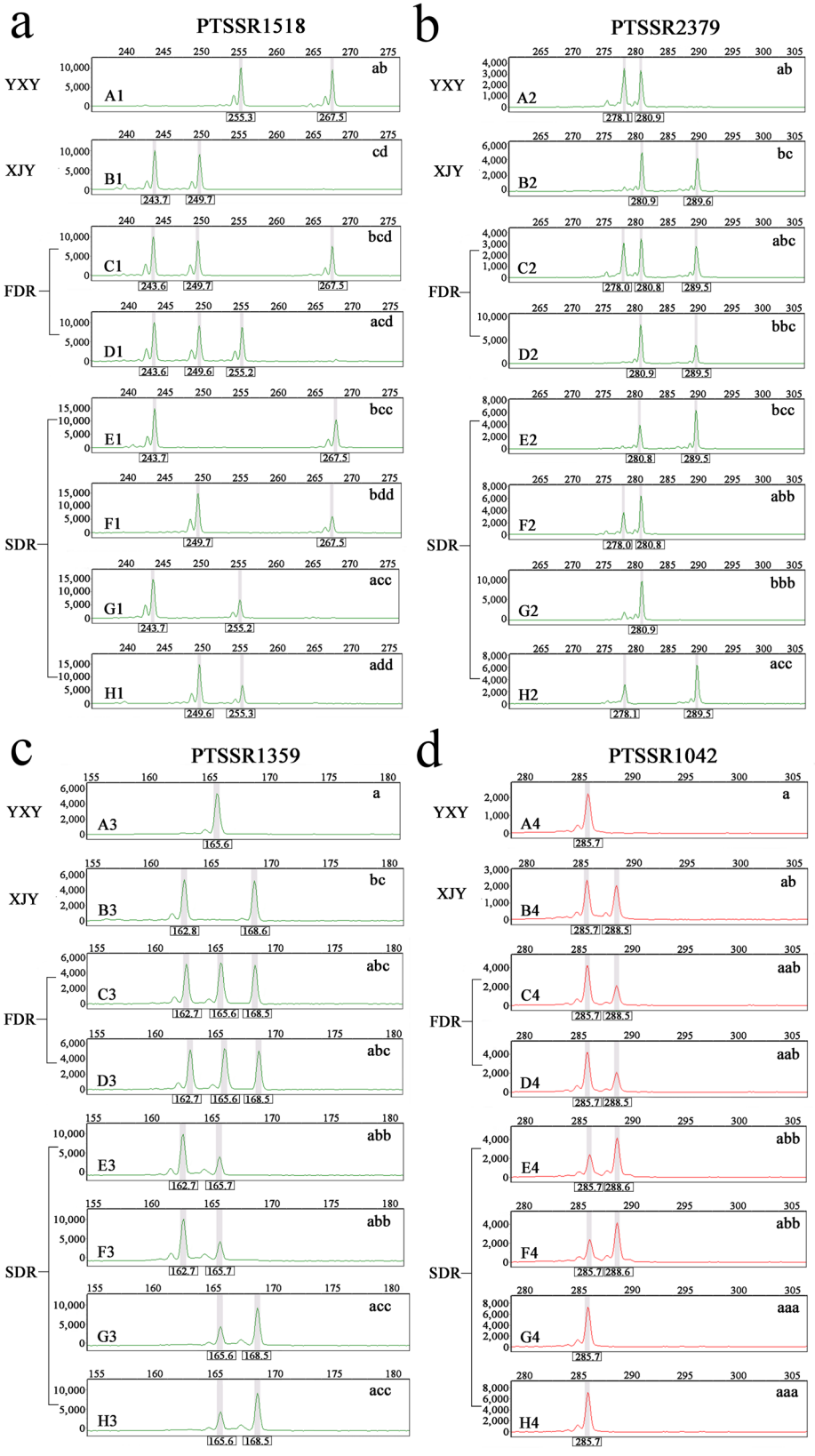

Fig. 4 (See legend on next page.)

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Fig. 4 Capillary electrophoresis was used for the SSR markers to infer the genetic composition in the two parents and their hybrid triploids. The genetic composition of each hybrid triploid originating from the cross combination between *P. alba* × *P. glandulosa* 'YXY' and *P. bolleana* 'XJY' was classified into an FDR-type or SDR-type. Heterozygous alleles from the male parent 'XJY' were fully transmitted to FDR-type hybrid triploids, whereas in SDR-type triploids, only one allele from the paternal heterozygous loci was inherited, and it typically appeared at double dosage, as indicated by increased peak height. (A) Female parent *P. alba* × *P. glandulosa* 'YXY'. (B) Male parent *P. bolleana* 'XJY'. (C-H) Six triploid progeny.

Transmitted parental heterozygosity by GA_3-induced 2n gametes

Based on the inferred transmission of parental heterozygosity in the GA_3-induced 2n gametes, the minimum heterozygosity transmitted by FDR-type and SDR-type 2n gametes was calculated as 0.5185 and 0.0000, respectively; their corresponding maximum heterozygosity transmitted was 0.8889 and 0.4815. The average parental heterozygosity transmitted by FDR-type 2n gametes was 0.7545, this far greater than that of SDR-type 2n gametes (0.3167) (Table 4). Furthermore, the FDR-type 2n gametes had far less variation in transmitted heterozygosity (CV = 12.82%) than the SDR-type 2n gametes (CV = 48.82%) (Table 4).

Discussion

The SSR markers with a high degree of polymorphism and codominance provide a reliable way to determine the genetic constitution of individual loci [12]. For *Populus*, Zhang et al. [28] reported that its 2n pollen originates from the SDR, while its 2n female gametes are derived from FDR, based on a small number of polyploid individuals with three pairs of SSR primers. Later, using 18 pairs of SSR primers, Liesebach et al. [17] analyzed the genetic composition of 2n gametes induced by chemical treatments in the *Populus*. Nevertheless, due to homologous recombination (HR) in meiosis, using only a small number of random SSR markers to assess the genetic composition of hybrid triploids could lead to inaccurate results [16]. Therefore, either a large number of random SSR markers [29–31] or a small number of SSR markers with low recombination frequencies are needed to robustly identify the genetic constitution of 2n gametes [32–34]. In our experiment here, 27 pairs of primers with adequate polymorphism were chosen from 193 candidate pairs of primers and used to analyze the genetic composition of 110 hybrid triploids. This revealed that 70 and 40 plants originated from FDR-type and SDR-type 2n gametes, or 63.64% and 36.36% of the total hybrid triploids, respectively. These results demonstrate that the GA_3 treatment produces more FDR-type individuals. However, a drawback of this approach is a long time needed to screen suitable polymorphic SSR markers.

Because 2n gametes are derived from different genetic constitutions, the transmitted heterozygosity is expected to differ depending on the particular 2n gametes involved [15]. In the absence of HR interference, the transmitted heterozygosity of FDR-type 2n gametes is 100%, compared to 50% for SDR-type 2n gametes; however, HR can complicate the transmitted parental heterozygosity by 2n gametes [35, 36]. Molecular marker technology is an accurate and convenient technique for calculating the transmitted parental heterozygosity by 2n gametes. When RFLP and RAPD markers are used to estimate transmitted parental heterozygosity by 2n gametes, reports have found that FDR is 1.50 to 2.25 times more effective than SDR in transmitting heterozygosity to progeny [15, 37]. Other studies found similar results for transmitted parental heterozygosity for FDR-type and SDR-type 2n gametes when using SSR markers [17, 18, 24]. In this study, we first estimated the transmission of parental heterozygosity in GA_3-induced 2n gametes, using 27 pairs of SSR primers. The results uncovered a significant difference in heterozygosity between the FDR-type (0.7545) and SDR-type (0.3167) 2n gametes. These findings are thus consistent with previous studies. Therefore, the differential transmitted heterozygosity of FDR-type vis-à-vis SDR-type 2n gametes stems from HR distinctions between homologous chromosomes of different individuals [16]. Although the 27 SSR primers used in this study were informative and sufficiently polymorphic, they covered only 9 out of the 19 *Populus* chromosomes. A higher-resolution assessment of transmitted heterozygosity would benefit from using more primers evenly distributed across all chromosomes.

Also, the FDR-type and SDR-type 2n gametes had large differences based on their CV value calculated from the heterozygosity transmitted by the 110 hybrid triploids. Relative to SDR-type 2n gametes (CV = 48.82%), there was little variation in heterozygosity transmitted by FDR-type 2n gametes (CV = 13.50%). This disparity is likely because during chromosomal crossover at a specific locus, the FDR-type 2n gametes have only a 50% probability of converting heterozygous loci to homozygous ones, whereas the SDR-type 2n gametes have a 100% probability of converting loci to heterozygous ones.

Differences in the genetic composition and HR of 2n gametes can lead to a significant divergence in phenotypic traits between the progeny of FDR-type triploids and SDR-type triploids [14, 24, 37]. Several studies have suggested FDR progeny may be particularly useful because FDR 2n gametes transmit more parental heterozygosity and the greatest parental epistasis to offspring [8, 17, 38]. Hansson and Westerberg [39] reported that higher heterozygosity could reduce the occurrence of duplicated homozygous genes, which would augment

organismal adaptation to the environment. Accordingly, FDR-type 2n gametes should be preferred over SDR-type 2n gametes in triploid breeding.

Conclusion

It is well known that triploids is a major force in plant evolution and diversification. Evaluating the transmission of heterozygosity by 2n gametes with different formation mechanisms is of crucial importance. we screened 27 pairs of SSR markers to evaluate the genetic composition and heterozygosity of induced 2n pollen grains. A total of 110 triploids were obtained by pollinating the female inflorescence of *P. alba* × *P. glandulosa* with GA_3-induced 2n pollen of *P. bolleana*. Among the 110 triploids, 70 triploids derived from FDR-type 2n pollen and 40 triploids derived from SDR-type 2n pollen. Notably, the FDR-type and SDR-type 2n pollen transmitted a substantially different level of parental heterozygosity (0.7545 and 0.3167, respectively) in *P. bolleana*. Additionally, compared with the low coefficient of variation (CV = 12.82%) for heterozygosity transmitted by FDR-type 2n pollen, there was much higher coefficient of variation (CV = 48.82%) for that transmitted by SDR-type 2n pollen. Therefore, our findings provide a valuable reference for the applications of 2n gametes in future studies, with a view to helping devise new and better strategies for triploid breeding in woody plants.

Table 3 Results for the microsatellite genotyping of 110 hybrid triploids based on 27 SSR loci

Offspring number	Number of marker genotypes of 2n gametes		FDR or SDR	Ho
	Heterozygous	Homozygous		
1	19	8	FDR	0.7037
2	17	10	FDR	0.6296
3	17	10	FDR	0.6296
4	21	6	FDR	0.7778
5	12	15	SDR	0.4444
6	22	5	FDR	0.8150
7	4	23	SDR	0.1481
8	17	10	FDR	0.6296
9	19	8	FDR	0.7037
10	11	16	SDR	0.4074
11	6	21	SDR	0.2222
12	21	6	FDR	0.7778
13	11	16	SDR	0.4074
14	18	9	FDR	0.6667
15	23	4	FDR	0.8519
16	22	5	FDR	0.8148
17	21	6	FDR	0.7778
18	23	4	FDR	0.8519
19	8	19	SDR	0.2963
20	21	6	FDR	0.7778
21	11	16	SDR	0.4074
22	22	5	FDR	0.8148
23	24	3	FDR	0.8889
24	2	25	SDR	0.7410
25	0	27	SDR	0.0000
26	19	8	FDR	0.7037
27	23	4	FDR	0.8519
28	11	16	SDR	0.4074
29	22	5	FDR	0.8148
30	23	4	FDR	0.8519
31	21	6	FDR	0.7778
32	0	27	SDR	0.0000
33	22	5	FDR	0.8148
34	21	6	FDR	0.7778
35	21	6	FDR	0.7778
36	10	17	SDR	0.3704
37	10	17	SDR	0.3704
38	2	25	SDR	0.7410
39	18	9	FDR	0.6667
40	5	22	SDR	0.1852
41	22	5	FDR	0.8148
42	2	25	SDR	0.7410
43	24	3	FDR	0.8889
44	9	18	SDR	0.3333
45	17	10	FDR	0.6296
46	10	17	SDR	0.3704
47	23	4	FDR	0.8519
48	18	9	FDR	0.6667
49	16	11	FDR	0.5926
50	9	18	SDR	0.3333
51	12	15	SDR	0.4444
52	23	4	FDR	0.8519

Table 3 (continued)

Offspring number	Number of marker genotypes of 2n gametes		FDR or SDR	Ho
	Heterozygous	Homozygous		
53	23	4	FDR	0.8519
54	12	15	SDR	0.4444
55	24	3	FDR	0.8889
56	14	13	FDR	0.5185
57	18	9	FDR	0.6667
58	12	15	SDR	0.4444
59	23	4	FDR	0.8519
60	17	10	FDR	0.6296
61	23	4	FDR	0.8519
62	18	9	FDR	0.6667
63	10	17	SDR	0.3704
64	10	17	SDR	0.3704
65	21	6	FDR	0.7778
66	12	15	SDR	0.4444
67	24	3	FDR	0.8889
68	16	11	FDR	0.5926
69	24	3	FDR	0.8889
70	13	14	SDR	0.4815
71	17	10	FDR	0.6296
72	20	7	FDR	0.7407
73	13	14	SDR	0.4815
74	22	5	FDR	0.8148
75	21	6	FDR	0.7778
76	20	7	FDR	0.7407
77	24	3	FDR	0.8889
78	18	9	FDR	0.6667
79	10	17	SDR	0.3704
80	11	16	SDR	0.4074
81	21	6	FDR	0.7778
82	23	4	FDR	0.8519
83	18	9	FDR	0.6667
84	6	21	SDR	0.2222
85	4	23	SDR	0.1481
86	22	5	FDR	0.8148
87	22	5	FDR	0.8148
88	19	8	FDR	0.7037
89	19	8	FDR	0.7037
90	22	5	FDR	0.8148
91	1	26	SDR	0.3700
92	24	3	FDR	0.8889
93	20	7	FDR	0.7407
94	19	8	FDR	0.7037
95	22	5	FDR	0.8148
96	21	6	FDR	0.7778
97	11	16	SDR	0.4074
98	15	12	FDR	0.5556
99	12	15	SDR	0.4444
100	14	13	FDR	0.5185
101	21	6	FDR	0.7778
102	13	14	SDR	0.4815
103	19	8	FDR	0.7037
104	13	14	SDR	0.4815

Table 3 (continued)

Offspring number	Number of marker genotypes of 2n gametes		FDR or SDR	Ho
	Heterozygous	Homozygous		
105	20	7	FDR	0.7407
106	18	9	FDR	0.6667
107	1	26	SDR	0.3700
108	10	17	SDR	0.3704
109	13	14	SDR	0.4815
110	10	17	SDR	0.3704

Table 4 Descriptives of the transmitted heterozygosity by the FDR-type and SDR-type 2n gametes

Groups	Minimum	Maximum	Average	Standard deviation (%)	CV (%)
FDR	0.5185	0.8889	0.7545	9.67	12.82
SDR	0.0000	0.4815	0.3167	15.46	48.82

Acknowledgements
Not applicable.

Author contributions
PDZ designed the research. YFZ, YYX, BK, DLC, JHD, LXM, MLL, LL, YRS, DWC, HQL, YQZ, HJZ and XYK performed the research. YFZ analyzed the data and wrote the original draft. PDZ reviewed and edited the paper.

Funding
This research was supported by the National Key R&D Program of China during the 14th Five-year Plan Period (2021YFD2200105).

Data availability
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 31 March 2025 / Accepted: 30 May 2025
Published online: 07 June 2025

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